STRESS AMELIORATION DURING LIVE TRANSPORT OF FISH

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CERTIFICATE

Certified that the dissertation entitled "**STRESS AMELIORATION DURING LIVE TRANSPORT OF FISH**" is a record of independent bonafide research work carried out by **Mr. Mohammed Koya, K.** during the period of study from September 2001 to August 2003 under our supervision and guidance for the degree of **Master of Fisheries Science (Mariculture)** and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship, or any other similar title.

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I hereby declare that the thesis entitled "**STRESS AMELIORATION DURING LIVE TRANSPORT OF FISH**" is an authentic record of my own research work and that no part thereof has been presented for the award of any degree, diploma, associateship, fellowship or any other similar title.

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सारांश

सजीव ग्रूपर मछलियों के लंबी दूरी परिवहन के समय, तापमान में घटाव करके, पैकिंग से पहले निश्चेतक (अनस्तेशिया) देते हुए और ऑक्सिजन की क्षतिपूर्ति के द्वारा होनेवाला तनाव-सुधार इस अध्ययन का विषय है। परिवहन के समय तनाव के परिणाम-स्वरूप अतिजीविता पर प्रभाव जानने के लिए पानी गुणाता प्राचल जैसे विलीन ऑक्सिजन, अमोनिया और पीएच के साथ साथ सीरम प्राचल जैसे ग्लूकोस, क्षारीय फॉसफेट और संपूर्ण प्रोटिन का अध्ययन किया गया। अमोनिया और सीरम प्राचलों के बढ़े हुए स्तरों से और विलीन ऑक्सिजन और पीएच के घटे हुए स्तरों के कारण अध्ययन के लिए पालित जीवियों में मृत्युता देखा गया जो इस बात का सूचक है कि परिवहन के समय तनाव में सुधार लाने के लिए किया गया उपचार सफल है । पैकिंग के समय जल का तापमान 150°c सं 20°c , 100 पी पी एम सान्द्रता का लौंग तेल निश्चेतक और ऑक्सिजन, 700 ग्रामवाले ग्रूपर समूह को 24 घंटे तक के परिवहन के लिए आवश्यक पाया गया।

ABSTRACT

Experiments were conducted to evaluate the stress ameliorative effects of temperature reduction, pre-packing anaesthesia and packing with supplementary oxygen on long duration live transport of groupers. Survival, water quality parameters like dissolved oxygen, ammonia and pH as well as serum parameters like glucose, alkaline phosphatase and total protein were studied as indicative response of the transportation stress. Elevated levels of ammonia and all the serum parameters and lower levels of dissolved oxygen and pH leading to mortality observed in the control group indicate that the treatments succeeded in ameliorating the stress caused by the transportation procedures. Packing water temperature of 15°C to 20 °C, anaesthesia with 100ppm clove oil and oxygen packing are found essential for transporting live grouper of biomass 700g for period extending up to 24h.

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1 INTRODUCTION

Live fish trade is emerging as a major business venture in most of the tropical countries. Broodstock and seed are widely transported by road, sea, rail or air to various destinations. Air lifting of live aquatic animals, especially live lobsters, shrimp and mud crab for export market has considerably increased during the last few years. Finfishes like groupers and ornamental fishes are transported live since they fetch premium price in the market.

Live transport of fish is a stressful and traumatic procedure consisting of a succession of adverse stimuli including initial capture, loading, holding, packing, transporting, unloading and stocking; deterioration of water quality during transit may impose additional stress to the animals. Minimizing stress within tolerable limits is the basis of live transport of fish. A detailed understanding of the physiological behaviour of the animal in high stress and low stress environments will provide an insight into the manipulations required to improve the survivability and keep them in best condition during live transport.

A variety of methods are in use to reduce the adverse effects on fish during transport. Pre-transport starvation, chilled shipping water and anaesthetics are commonly employed to minimize metabolic activity of aquatic animals during transport. A standardized protocol for cost effective, low stress mode of transport for live fish for human consumption is not available at present.

Lack of adequate information on handling fishes after they are caught, right through the transport and sale to the customers, is a major constraint to the live fish trade. The present study has been taken up to understand the ameliorative measures to be taken to scale down the effect of stress during live transport of grouper, a high value fish in domestic and international market.

2 REVIEW OF LITERATURE.

2.1 Live transport

Aquaculture and live handling of fish have been practiced for centuries in many Asian countries. In fact, marketing of fish alive is a practice deep rooted in many South East Asian countries, but the trade was of localised nature restricted to the areas of production and their vicinity. However, large scale commercial operations of live fish handling was made possible only with the recent technological developments in live fish handling gear, efficient transportation and a better understanding of the biological aspects of live fish handling (Subasinghe, 1997).

In terms of the range of species and distances shipped, tropical fishes stand first in live fish transport (PeerMohammed and Devaraj, 1997) Critical biological criteria are to be met when shipping live finfish and shellfish, if not, they will all die quickly. Obviously, careful harvesting and post harvest handling is of utmost importance (Singh, 1991).

Presently there are no international regulations *per se* governing the movement of fish and fish products. Instead, the importer of fish faces a myriad of different domestic (national) regulations governing the import of fish and fish products (Kinkelin and Hedrick, 1991).

Concurrent with the increase in live seafood and aquarium fish trade the support service and technology provided has not improved in some of the major exporting and developed countries. IATA regulations for live air shipments have been in existence for some time. More airlines and relevant government support services are researching and drawing guidelines for the potential exporter (Richards-Rajadurai, 1989).

The Government of India as an extraordinary Gazette notification Part II – section 3- sub-section (ii) dated 1st May, 2002 published the rules for live fish export (quality control, inspection and monitoring).

2.1.1 Live seafood trade

As with many perishable products, consumers pay much attention to freshness while buying fish. Fish can not be any fresher than when it is live. Supplying the consumer live fish is, perhaps, the best method of ensuring that he is getting the fish fresh (Subasinghe, 1997). The demand for live fish and shellfish is rapidly increasing and in some industrially developed countries, more and more retail fish traders and restaurants are offering live fishery products to their high-income customers who can and do not mind paying considerably higher prices for live products than for fresh-iced products (Peer Mohammed and Devaraj, 1997).

Serving live fish in the restaurants is actually begun in Western Japan, a few decades ago (Kano, 1991). The tradition of *ikejime* meaning preparation of live fish for raw consumption after starving it for a few days based on the belief that fish taste best when eaten raw about twelve hours after being killed which also enables easier handling and transport, reduces the smell of feed and produces leaner meat is thus considered to be a major contributor to the popularization of live fish consumption in Japan (Kano, 1991)

With changing life style of people, the demand from many of the affluent consumer markets gradually started shifting towards live fish and shellfish and this is showing an increasing trend. Even though live transportation of fish, especially farmed freshwater fish was prevalent in Europe (Norris *et al.*, 1960), transport of several species of fish and shellfish of marine, brackishwater and freshwater origin to distant markets is relatively of recent origin. Affluence of consumers and scientific understanding and technological developments in handling and packaging techniques has provided added impetus to the prospects of live fish and shellfish for human consumption (Balachandran *et al.*, 2001; Singh, 1991). Marketing of live fish is regarded as value-adding procedure

because live fish fetches substantially higher prices than fresh-chilled or frozen products (Chan, 2000)

2.1.2 Live grouper trade

Groupers (Family, Serranidae) are widely distributed in the coastal waters of tropical and sub-tropical regions of all the oceans. They are mainly found in less than 100m deep waters.

Groupers are of economic value and form a major component of the coastal artisanal fisheries in the tropics. Groupers are one of the most expensive fishes in Asia, particularly in Hong Kong. One has to pay a premium price for cultured groupers and even more for wild caught live ones (Seng, 1998).

Live grouper being an expensive fish, is typically found in high class Chinese restaurants specializing in seafood. Hong Kong and southern part of China are considered as the main markets for live grouper. However, as a major portion of live grouper supplies to China comes from Hong Kong, the latter is obviously the main player in the live grouper trade. Other small markets include Taiwan and Singapore (Pawiro, 1999). Red coral trout, *Plectropomus areolatus*. is the most common medium-priced fish in both Hong Kong and Peoples' Republic of China having a whole sale price of about US$ 38/kg. Less expensive ones are green grouper, *Epinephelus malabaricus* and yellow spotted grouper *E. coioides*. (US$ 20/kg).

According to Census and Statistics Department (CSD) live marine fishes were consigned to Hong Kong from 21 exporting countries including India Maldives and ShriLanka with Thailand, Mainland China, Indonesia and Taiwan as the major exporting countries (Gilvray and Chan, 2002).

2.2 Methods of transportation

Many molluscs like clams, mussels, oysters and crustaceans such as some species of shrimp, crab and lobster and air-breathing fishes like murrel, can remain out of water for quite some time, if suitable environment is provided and can be transported without water (Balachandran *et al.*, 2001).

2.2.1 Crustaceans

For shrimp species like black tiger shrimp (*Penaus monodon*), giant fresh water prawn (*Macrobrachium rosenbergii*) *etc* a closed system or an open tank system using aerators can be conveniently used for relatively short journeys. Broodstock and post larvae of shrimp are transported live employing the closed plastic bag system for inland transportation or for air transport overseas (Balachandran *et al.*2001; Richards-Rajadurai, 1989).

Kuruma shrimp has been transported live in a state of cold-induced hibernation in chilled saw dust, making it the most expensive shrimp in Japan (Shigueno, 1992).

In a pioneering study, Salin and Jayasree-Vadhyar(2001) revealed that black tiger shrimp *Penaeus monodon* could also be used for live storage by cold anaesthetization in saw dust under conditions similar to those used for kuruma shrimp *P japonicus*.

Healthy and conditioned lobsters pre chilled to 4°C are packed in polystyrene boxes laid with ice pads and impregnated with silicon to absorb melt water. They are packed in layers separated by moistened materials such as seaweeds, wood shavings or similar materials to maintain the relative humidity at 70% and temperature at 1-7°C (Balchandran *et al.*2001; Richards-Rajadurai, 1989).

2.2.2 Molluscs

Transportation of mussels at 2-4°C separated from ice by a layer of perforated material such as cheesecloth with constant re-icing in insulated containers is reported by Shoemaker (1991). Commercial air shipment of pre-chilled live oysters is usually in polystyrene boxes or waxed cartons with inner polyethylene liners. Other packaging materials include barrels, sacks or specially compartmentalized containers, depending on the value of the product and the market destination (Richard-Rajadurai, 1989).

2.2.3 Finfishes

Large quantities of fish may be transported using 'live well-boats' and large containers placed on trucks. Live market sized fish also may be transported in oxygenated plastic bags when the shipment is small, or when air travel is involved (Shoemaker, 1991; Balachandran *et al.*, 2001)

Fish are generally transported in containers such as cans of different sizes, pots of ceramic or metal, wooden or metal buckets, vats, barrels, plastic bags, styrofoam boxes, bottles, jugs, animal skins and bamboo sections. In fact, almost any clean, waterproof container may be used. Certain containers provide good insulation from heat, for example, wood or styrofoam. Containers like metal and plastics are poor insulators and may have to be wrapped with wet towels or packed with ice to keep temperatures down (Rimmer and Franklin, 1997).

Packing fish in the sealed polythene bags or plastic containers partially filled with water and air is the most basic method of packing live fish. Even though the method is used for the fry and fingerlings, it could also be used for transport of market size fish as well, for short periods of time (Subasinghe, 1997). Fish are packed in plastic bags that are inflated with pure oxygen, closed with rubber bands, placed in an insulated corrugated box and sealed. The size and shape of these bags and box as well as the insulation can vary widely (Richards-Rajadurai, 1989).

According to Rimmer and Franklin (1997), three basic methods that are commonly used in Australia for transporting live fish by air are the polystyrene box, the 'pickle barrel' system and the 'big box' system. Australian live-fish exporters use insulated plastic bins having one-tonne water capacity with battery-operated air pump fitted at the upper compartment of the bin to inject air into the water through a diffuser to transport 240 kg of live fish by air to Hong Kong with good survival rate if delivered to the buyer within 24 hours. However returning the bins is costly (Rimmer and Franklin, 1997).

Specially-designed containers used in successful transportation of live seabream by rail, boat and air from New Zealand to Japan by a few major marine transport companies (Ichiro Kano, 1991).

The impetus for the development of safe techniques for live transportation of fishes came from candid observations that often the entire shipment of fish would die during or soon after transport. Fish losses related to transport events are especially critical because the shippers are generally had full investment in the animals at the time of harvesting and transporting. Losses associated with transport also interrupt stocking and processing schedules (Carmichael *et al.*,2001).

Fish are the most difficult live aquatic animal item to be transported by air. But with good practices their survival rate is very good if the total transportation time is within 24 hours (Chan, 2000).

About 3Kg of fish, which has been conditioned, can be packed in 10-12 litres of chilled water and ideally, an equal volume of head space filled with oxygen (Subasinghe, 1997).

2.2.4 Shipping Bags

Pleated bags (flat bottom bags) utilize the entire surface area of the box allowing maximum oxygen transfer through the surface of the water and reduce crowding by utilizing all the available area in the box (Cole *et al.*, 1999).

Fish packers in Asia generally use bags manufactured from stock tube plastic and heat-sealed at one end so there is a single seam and these are called the "pillow bags" in the ornamental fish industry, because when they are inflated there is no flat surface, and packing water surface is increased by shipping them on their side (Cole *et al.*, 1999).

2.2.5 Polystyrene box

The most commonly used type of container is polystyrene box (approximately 50x50x50cm dimension and thickness >3cm) packed in an outer corrugated fibre board box. Ornamental fish packed from Asia frequently make use of one of two size boxes, 60cm (L) x 42cm (W) x 30cm (H) or 49cm (L) x38cm (H) x38cm (H). Both sizes are packed with a minimum of four bags (Cole *et al.*, 1999).

Purpose built aerated fish transport boxes made of fiberglass or HDPE can hold more fish per unit volume, a fish: water ratio of 1:1 when compared to a ratio of 1:3-4 attributed to polystyrene boxes. One such container is the Australian built DYNO Live Transporter, a modular container which has been specifically designed for air carriage of live seafood in water (Subasinghe, 1997).

2.2.6 Advanced methods of fish transportation

Several advanced methods of transporting live fish are practiced in different countries. The species so transported are mostly high value species of fish destined to lucrative markets. These methods employ techniques such as the use of very low temperatures, chemical oxygen supply restriction of body movements and even virtual "freezing".

In a system designed by a New Zealand company, snapper is air lifted to Japan in styrofoam boxes in a few ounces of water recirculated and kept chilled by a micro refrigerator. The patented battery operated recirculation unit is coupled to a chemical oxygen supply system. In this system, 4 snappers of 1-2Kg each with a gross weight of under 20Kg.

Japanese scientists have also introduced a method which virtually "freezes" the fish prior to air travel. This method of water-free transportation is applicable to fishes with flat body such as sole. I n the method used for sole, fishes held in the seawater tank is covered with nets and the water in the tank is cooled by a refrigeration system to freezing point. At this, stage the water is

drained and fish packed for air transportation. The fishes are revived at destination by immersing in water at room temperature. Trans Atlantic transport of 34 hours was possible by this method.

Several other methods, such as cooling case system, partial paralysis of fish by severing the connection of spinal cord from the brain *etc*, are reviewed by Subasinghe (1997).

2.3 Problems in live fish transportation

2.3.1 Metabolic rate and oxygen depletion

Some of the several problems encountered in the transport of live food fishes are: low solubility of oxygen in water and its poor capacity to dissipate the end products of metabolism, sensitivity of the fish to handling, accumulation of dangerous levels of lactic acid in the blood and muscle due to capture and handling, excessive changes in the temperature from catching to handling and live carriage *etc.* (Balachandran *et al.*, 2001).

Changes in water quality can result in high shipping mortalities during transport of fishes in sealed containers. A decrease in the concentration of dissolved oxygen and an increase in acidity and in the concentration of carbon dioxide and ammonia are the most potentially harmful of these changes (Nemoto, 1957; Mc Farland and Norris, 1958; Braker, 1974, Chan, 2000, Rimmer and Franklin, 1997).

The major obstacle in live fish trade is the transport cost of water. To carry 1-2 tones of fish, some 10 tonnes of water had to be carried with it, thus keeping the price very high (Subasinghe, 1997)

The key to successful transport is to keep transport time to a minimum and reduce stress from handling, crowding, changes in temperature, low oxygen, and elevated ammonia. (Rimmer and Franklin, 1997).

2.3.2 Ammonia and Carbon dioxide

In teleosts, ammonia is the main nitrogenous waste material produced from the catabolism of amino acids, purines and pyrimdines (Randall and Wright, 1987)

The rate of excretion of ammonia is related to the rate of metabolism and size of the fish. Large fish of a given species produces less excretion products than do the smaller ones of the same species and are also more resistant to ammonia than the smaller ones (Balachandran *et al.*, 2001)

The effect of increase in carbon dioxide is to depress the ability of active fish to take up the oxygen. Accumulation of free carbon dioxide up to 25ppm can be tolerated by a sensitive species under adequate aeration, and up to 50ppm by intensive aeration (PeerMohammed and Devaraj, 1997)

Exposing fish to unusually high carbon dioxide or ammonia levels cause plasma pH to fall to near lethal levels. The effects of both of these wastes need to be considered when studying the responses of fish to live transport (Rimmer and Franklin, 1997)

Two methods are commonly used to control the accumulation of ammonia in transport water: preventing ammonia formation by altering the metabolism of the fish and by removing ammonia from the water after it has been excreted. Some recommended methods for decreasing the metabolic rate (and hence the rate of ammonia excretion of confined fishes) are lowering the water temperature (Phillips and Brockway, 1954; Voss, 1979), adding anaesthetics to the water (Phillips and Brockway, 1954; Nemoto, 1957; Mc Farland, 1960; Murai *et al.*, 1979), and withholding fish for an extended period before the fish are shipped (Phillips and Brockway, 1954; Van den Sande, 1974)

Two principal methods of removing ammonia in water are: (1) nitrification, and (2) ion exchange. Nitrification is a two-step oxidation of ammonia to nitrite by autotrophic bacteria, and is an essential part of a recirculatory system (Bower and Turner, 1981). Turner and bower (1982) examined the influence of

bacterial nitrification during the transport of live fish and showed that addition of a substrate containing nitrifying bacteria to a sealed plastic bag is a practical way of reducing ammonia.

Adequate concentration of dissolved oxygen commonly are maintained by displacing the air in transport containers with pure oxygen, and organic and inorganic buffers have been used to prevent acidification of the water and accumulation of carbon dioxide(Nemoto, 1957; Mc Farland and Norris, 1958; Braker, 1974).

2.3.3 pH

The most important effect of pH on aquatic animals is related to changes from non-toxic ammonium ion to toxic ammonia (as the dissociation of ammonia is controlled by pH). For pH <8 there is only ammonium ions which become ammonia at elevated pH (>8.5). pH of water between 6.5 and 8.5 is ideal for most fish. High pH and low pH are detrimental to fish (Rimmer and Franklin, 1997).

The fraction of excreted ammonia which appears in the toxic form is markedly affected by the presence of acids in the water. The precise quantity of unionize ammonia associated with a given pH varies with temperature; it is higher at higher temperatures (PeerMohammed and Devaraj, 1997)

2. 4 Stress in fish

The concept of biological stress applied to fishes has attracted considerable attention in recent years. While many of the earlier studies addressed the effects of stress on cold water fishes such as salmon (*Oncorhynchus* spp.), and trout (*Salmo* spp.) (Barton and Peter, 1982; Schreck, 1982), recent studies have investigated sub-tropical species such as snapper *Pagrus auratus* (Lowe *et al.*, 1993) red drum *Scianops ocellatus* (Robertson *et al.*, 1988) and sea bream *Sparus aurata* (Arends *et al.*, 1999). Generally these investigations have sought to provide information aired at enhancing aquaculture

production or explaining fish responses during capture and transport (Carragher and Rees, 1994, Davis and Parker, 1986).

A tropical reef fish which has been studied for its responses to stressors like capture, handling, transport and shallow water stress is the blue spotted coral trout, *Plectropomus leopardus* (Frisch and Anderson, 2000).

Stressors that elicit profound responses in mammals may not result in comparable physiological changes in fish, and *vice versa*. .Even among fish species there is considerable variation in the levels of response, even within the same species, it may vary with season and stage of development (Reddy and Leatherland, 1998).

Blood is the most accessible element of the teleostean body fluid system to study the stress response. Consequently, blood variables are commonly used as direct or inferential indicator of functional state, the validity of which rests in however, on several assumptions; most importantly that neither the sampling procedure nor the subsequent sample treatment significantly alters *in vivo* characteristics (Korkock *et al.*, 1988)

2.4.1 Stress in live transportation

Carmichael *et al.* (2001) divided stressors causing mortality during transport or delivery of fish in poor condition in to severe stressors (physical or chemical agent that causes the pain or discomfort) and mild stressors (shaking as the transport vehicle moves, crowding and less than optimum water quality (high ammonia levels, high carbon dioxide levels, low dissolved oxygen concentrations or extreme temperatures) or infectious diseases (usually occurring one or two weeks after transport) induced by the stress associated with transport techniques. Severe stressors associated with harvesting and transporting that may kill fish in a short period of time are usually actions associated with inadvertent mismanagement of harvesting and transporting activities

One common mistake is concentrating fish (either by seining or draining the ponds or tanks) in a small volume of water without adequate aeration

causing animals to die from hypoxia. Other examples of severe stressors include movement of fish from water of one temperature to water of very different temperature, crushing fish by loading dip nets or boom baskets too heavily (Carmichael *et al.*, 2001)

2.4.2 Stress response in fish

To date, most of the information on stress response of fish are derived from studies on salmonids (Sumpter, 1997) and a range of temperate, non-salmonid species (Pankhurst and Sharples, 1992; Vijayan *et al.*, 1993; Sunyer *et al.*, 1995 and Bernett and Pankhurst, 1998) but with a few exceptions, (Pankhurst *et al.*, 1997 and Frisch and Anderson, 2000) there is very little information on the stress response of tropical species of fishes.

The response of fish to stress can be divided into primary, secondary and tertiary components, according to the level of organization at which it operates (Wedemeyer and Mc Leay 1981; Wandelar Bonga, 1997) The primary response to stress involves the activation of two major systems: the hypothalamic-pituitary-interrenal (HPI) axis and the sympathetico chromaffin (SC) system. Stimulation of HPI axis results in increased circulating levels of cortisol while stimulation of the SC system results in increased circulating levels of adrenaline. The neuroendocrine reactions in turn stimulate secondary responses which are manifested as changes in a range of biochemical, physiological, hematological and immunological parameters and in general, magnitude of the stress response reflects the intensity of stress experienced (Barton and Iwama, 1991).

2.4.2.1 Catecholamine

Adverse stimuli cause hyper secretion of catecholamine and corticosteroids in teleosts which in turn induces a suit of secondary effects including osmoregulatory, metabolic and immune system disturbances (Mazeud *et al.*, 1977). Measurement of these parameters has been used successfully to assess the degree of trauma caused by exposure to adverse stimuli (Wedemeyer

assess the degree of trauma caused by exposure to adverse stimuli (Wedemeyer and Mc Leay, 1981), including transport procedures (Barton *et al.*, 1982; Tomasso *et al.*, 1980; Davis and Parker, 1986).

Mazeaud *et al.*, (1977) reported that the stressors, struggling, hypoxia, temperature, capture and transportation in sock eye salmon and chinook salmon resulted in significant increase in the level of plasma catecholamine and corticosteroid when measured. However the quantitative response depended on the type of stress.

2.4.2.2 Cortisol

Cortisol is released by the interrenal tissue due to the action of the pituitary hormone, adrinocorticotropin (ACTH) on corticosteroidogenic cells. Similarly as in mammals, cortisol secretion down-regulates the activity of the hypothalamus-pituitory-intrrenal (HPI) axis, by negative feed back on the hypothalamus (Fryer and Peter, 1977).

The elevation of plasma corticosteroids mainly cortisol, in response to various types stressful stimuli in salmonid fishes has been well documented (Iversen *et al.*, 1998; Barton, 2000). Frisch and Anderson (2000) claimed that the slower and lesser cortisol response to the controlled stressors compared with wild capture, handling, and transport reflect the relative intensity of these stressors.

Anaesthesia, crowding, confinement, handling, temperature, transport, electroshock and dewatering are stimuli that have been shown to cause significant rise in cortisol levels, characteristic of the primary response in salmonid fishes (Flodmark *et al.*, 2001).

Flodmark *et al.* (2001) in an experiment meant to study the response of the juvenile brown trout to fluctuating flow regime in an artificial stream showed that the blood plasma cortisol concentration reached a peak in two hours of down ramping and returned to basal levels in 6 hours time.

Robertson *et al.* (1988) reported that the initial procedures in transportation, including capture, loading, and the onset of transport, induce

transported with anaesthetic MS-222 (5mg L^{-1}) also showed elevated levels of cortisol.

Frisch and Anderson (2000) suggested that the plateau formation in the cortisol response in coral trout *Plectropomus leopardus* subjected to a single stressor is due to the negative feed back of cortisol on the adreno corticotrophic hormone (ACTH) release from the pituitary (Barton and Iwama, 1991).

Along with primary adrenergic and cortisol response, secondary and tertiary responses such as elevated blood glucose concentrations, suppressed levels of thyroid hormones, growth hormones and electrolyte and altered behavior are evident (Pickering, 1992; Mc Donald and Milligan, 1997; Pankhurst and Van Der Krack, 1997).

Examples of stressors are hauling, sorting and transport. A classic stress response involves the activation of two components in the neuro-endicrine system, the adrenergic system (adrenaline and nor adrenaline) and the hypothalamo-pituitary axis (cortisol) (Selye, 1950).

Fish respond to stress with a characteristic acute increase in plasma levels of the catecholamines, adrenaline and noradrenalin, and slower but more sustained increase in plasma levels of the corticosteroid cortisol (Sumpter, 1997).

Iversen *et al.* (1998) showed that, regardless of size/age and strains, the overall response to hauling and transport was similar: increased plasma cortisol, lactate, and glucose levels with corresponding changes in haematocrit, and a severe disturbance in hydromineral homeostasis both in fresh water and sea water.

2.4.2.3 Glucose

Three physiological changes routinely found in stressed fish are the elevations of plasma cortisol, glucose and lactate concentrations (Nikinmaa *et al.,* 1984).

Increase in the catecholamines and corticosteroid levels are generally mirrored by an increase in plasma levels of glucose generated by the glucose-mobilizing effects of both classes of hormones (Barton and Iwama, 1991; Wandelaar Bonga, 1997).

Metabolic disturbance due to endocrine action during stress include profound increase in blood glucose and a variation in plasma free fatty acids according to the species. These changes brought about by stress of short duration are shown to be relatively long lasting. (Mazeaud *et al.,* 1977).

Recent advances in the study of stress responses in fish have shown that blood glucose, lactate, and plasma protein values correlate with a wide range of stressors (Korcock *et al.*, 1988, Robertson *et al.*, 1988; Pankhurst and Dedual, 1994

Hyperglycaemia is a commonly reported stress response in fish (Braley and Anderson 1992; Specker and Schreck, 1980)

Post-stressor increase in blood glucose levels has also been used as indicators; glucose is easy to measure, relatively inexpensive, and the most commonly used indicator of the secondary stress response in fish (Wedmeyer *et al.*, 1990)

Transportation procedures have shown to increase blood glucose in several fish species. Transport induced hyperglycaemia has been reported for small mouth bass (Carmichael *et al.*, 1984), in large mouth bass (*Micropterus salmoides*) (Carmichael *et al.* 1984), in red drum (*Scianops ocellatus*) (Robertson *et al.* 1988) and in matrinxa (*Brycon cephalus*) (Carnairo and Urbinati, 2002)

Transportation procedures induced rapid elevations in plasma cortisol and glucose concentrations (100-160 $ng.ml^{-1}$ and 40-60 mg per 100ml, respectively) within 15-30 minutes of capture and loading in cultured red drum. Thus capture and loading are most traumatic procedures involved in its live transportation (Robertson *et al.* 1988).

Harvesting and processing such as capture, handling, confinement and transportation frequently cause stress-induced reduction in stock quality those are attributable to the effects of secondary responses such as hyperlactecemia and tissue catabolism (Lowe *et al.* 1993)

2.4.2.4 Lactate

Lactate is produced by anaerobic metabolism in the white muscle under conditions of hypoxia or strenuous exercise (Driedzic and Kicenule, 1976). Frisch and Anderson (2000) opined that the elevated lactate levels in coral trout *P. leopardus* could be due to the struggling during capture and due to the anaerobic metabolism resulting from the emersion during hook removal and air bladder deflation.

Swimming challenge increased the plasma lactate concentration from 0.6 m mol l^{-1} in stressed striped bass to between 10 and 25 m mol l^{-1} (Strange and Cech, 1992).

Blood lactate was high in all the game fishes stressed during capture compared to the unstressed snappers used as controls (Wells *et al.*, 1986).

2.4.2.5 Free Fatty Acids

In a carp, a two hour hypoxia and a forced swimming until exhaustion showed a decrease of free fatty acid level and an increase in glucose level in the blood (Mazeaud, 1977).

2.4.2.6 Proteins and enzymes

Wells *et al.* (1986) showed that there is stress related increase in plasma albumin and total protein.

Plasma enzymes are useful indicators of extreme stress and may give specific information about organ dysfunction. Useful indicators might be alkaline phosphatase, aminotransferases and possibly lactate dehydrogenase (Wells *et al.*, 1986)

2.4.2.7 Haemoglobin

Hematological techniques, including measurement of hematocrit, erythrocyte and leukocyte counts, have proven valuable for fisheries biologists in assessing the health of the fish (Blaxhall, 1972; Hickey, 1976) and in monitoring stress responses (Swift and Lloyd, 1974)

Wells (1986) reported that high hemoglobin in stressed snapper fell to lower levels during recovery. Haemoglobin and hematocrit were very high in teleostian game fishes except for blue marlin after capture stress.

Frisch and Andersen (2000) indicated that both erythrocyte release and swelling occurs in response to stress associated with capture, handling, shallow water and transport. Hematocrit was significantly elevated at 15 minutes and a maximal at 30 minutes after capture from wild and in response to shallow water stress.

2.4.2.8 Leucocytes

The number of leucocytes and thrombocytes in the circulating blood of fishes seems to reflect more accurately than the number of erythrocytes the fish's reaction to acute stress, including that caused by pollution.

McLeay and McGordon (1977) introduced a test, leucocrit for estimating the circulating levels of leucocytes because of its rapidity, sensitivity, and reliability in indicating the stress response.

2.4.2.9 Electrolyte and water balance

Capture stress has profound effect on salt balance in seawater fish resulting in elevated sodium chloride levels and osmolality (Eddy, 1981). Serum osmolality increases following capture of marine teleosts (Umminger, 1970) and in sharks for three hours post-capture (Cliff and Thurman, 1984)

Plasma sodium, potassium, chloride, calcium, iron and inorganic phosphate concentrations were higher in capture stressed game fishes than in unstressed snappers (Wells et al. 1986)

2.5 Stress amelioration

To avoid stress and mortality during transportation, Carmichael *et al.* (2001) identified some general guidelines for harvesting and transporting fish.

2.5.1 Temperature reduction

Water temperature is an important factor as it determines the dissolved oxygen concentration. The lower the temperature, higher is the oxygen saturation and lower is the oxygen consumption by fish. Water temperature also decides the stocking density. For each 10°F decrease in temperature, loading density can be increased by 25% for channel cat fish and other warm water species like carps *etc.* (Little, 2001).

Water can hold more oxygen in solution at low temperatures; however, fish requires more oxygen at higher temperatures. Therefore, a tank of a given volume can hold more fish at lower temperatures than it can hold at high temperatures. That is the reason why the temperature of water in transportation is always kept low according to the levels that the fish concerned can tolerate (Balachandran *et al.*, 2001).

2.5.2 Lowering of metabolic rate

Discharge of metabolic waste may be controlled by lowering the metabolic rate of fish and using suitable substances to remove them. Reduction in metabolic rate can be achieved by lowering the temperature, addition of

anaesthetic to water and through conditioning of the fish. (Balachandran *et al.*, 2001).

It is well known that metabolic rate is influenced by environmental factors, particularly temperature and dissolve oxygen concentration (Mallekh and Lagardere, 2002). Fry (1971) considered temperature to be a factor controlling metabolic rate and the latter to be a limiting factor.

Most temperature reduction treatments improved water quality. Best survival (89%) of barramundi (*Lates calcarifer*) during live transportation was achieved by reducing water temperature by 10°C at slow cooling rate of 5°C per hour(Rimmer and Franklin, 1997).

While transporting fish live, lowering of water temperature tends to decrease the metabolic rate of the animal and thus decrease the amount of ammonia excreted in to the water. In addition, lower temperature reduces the requirement for oxygen (Cole *et al.*, 1999).

2.5.3 Anaesthetics

The first worker to recognize the potential use of anaesthetics for transporting fishes appears to have been Aitken (1936).

When choosing an anaesthetic, a number of considerations are important: efficacy, cost, availability, ease to use and side effects on fish, humans and environment (Marking and Meyer, 1985).

The use of anaesthetics in fisheries and aquaculture research greatly facilitates many procedures including induction of spawning, obtaining body length and weight, conducting gonadal biopsies and transport (Soto and Burhanuddin, 1995).

With the use of anaesthetic, it is possible to increase loading density of fish distribution units. Also the tranquilising effects of anaesthetics reduce injury to large or excitable fishes when they are transported. (Little, 2001)

Deep sedation which suppresses the reactivity of fish to external stimuli without upsetting equilibrium and which reduces oxygen consumption to basal rate seems best suited for transporting fish (Mc Farland, 1959, 1960).

Fishery biologists use anaesthetics to reduce handling stress on fish. The use of some anaesthetics on food fishes, however, is limited. Chemicals and compounds traditionally used by aquaculturists are now under stringent scrutiny, not only for cost effectiveness but also for safety and efficacy (Taylor and Roberts, 1999)

Marking and Meyer (1985) defined several criteria for an ideal anaesthetic noting that probably no compound will fulfill all criteria. Their criteria were rapid immobility, quick recovery, no toxicity to fish, no mammalian safety problems, low tissue residue and low cost (Marking and Meyer, 1985).

2.5.3.1 Metomidate

Metomidate, dl-l-(1-phenylethyl)-5-(methoxycarbile) imdazole hydrochloride, and the closely related compound etomidate, are rapid acting, non-barbiturate hypnotics in several species. Metomidate is presently marketed as a fish anaesthetic. The anaesthetic has a short induction time in fish, although the recovery time is rather longer (Gilderhus and Marking,1987)

2.5.3.2 MSS-222

At present, the only anaesthetic cleared by the US Food and Drug Administration (FDA) for aquaculture use in the United States are tricaine methaine sulfonate (MS-222) and carbon dioxide (CO_2) (Schnick *et al.*, 1986): MS-222 has a 21 day recovery period before fish can be consumed (Anderson *et al.*, 1997) and carbon dioxide does not produce sufficient anaesthesia, and fish have a tendency to react violently after application (Taylor and Roberts, 1999)

Anaesthesia with MS-222(80mg/l) prior to capture and, coupled with the use of sedating doses of (5 and 25mg/l) MS-222 in the transport tanks did not decrease the magnitude of the response. On the contrary, the

corticosteroid stress response was augmented in fish transported in 25mg/l MS-222 (Robertson *et al.*,1988).

Acute exposure to immobilizing doses of an anaesthetic, MS-222, is an effective method of reducing endocrine and metabolic disturbances during transportation of red drum (Robertson *et al.*,1988) Most of the anaesthetics that are used as non-destructive chemical based sampling method for sampling the rock pool fish assemblages like quinaldine and MS-222 *etc* are expensive and can present a health hazard to the researcher. (Anderson *et al.*, 1997).

2.5.3.3 Clove oil

Alternative anaesthetics on food fishes which are effective and have short or no depuration period, would fulfill a priority need in fisheries science (Gilderhus and Marking, 1987). One such promising anaesthetic is clove oil, a distillate of herbatious portion of the clove tree *Eugenia aromatica* containing the active ingredient eugenol 90-95 %(Brizzo *et al.*, 1989).

Clove oil has a long history as a local anaesthetic for humans (Soto and Burhanuddin, 1995). It is considerd non-mutagenic and a safe substance by the FDA (Nagababu and Lakshmanaiah, 1992), with human intake levels established at 2 – 5 mg /Kg/day (Expert committee on Food Additives, 1982).

Clove oil whose active principle is eugenol (4-allyl-2-methoxy phenol) is derived from the stem, flowers, and leaves of clove trees (*Eugenia caryphyllata* and *Eugenia aromatica*). Eugenol is a highly versatile chemical used in a range of applications such as an antifungal and antbacterial agent (Griffith, 2000 and Soto and Burhanuddin, 1995). Clove oil is a topical anaesthetic cleared for use in human medicine and dentistry by the FDA (Nagababu and Lakshmaniah, 1992).

Soto and Burhanuddin (1995) first evaluated clove oil as an anaesthetic for rabbit fish, *Siganus lineatus* Cuvier and Valenciennes, and found that both rapid induction (108 sec) and recovery (76 sec) times at concentration of 100 mg/l.

Clove oil has been used for a number of years to anaesthetize fish in seawater. which is essential in some basic procedures in fish farming such as weighing, tagging experimental work and for transport. It considerably reduces the pathology risks from stress, injury and accident during handling (Keene *et al.*, 1998).

There is a growing interest in the use of clove oil as a fish anaesthetic (Griffith, 2000). It is effective for anaesthetizing rainbow trout, *Oncorhynchus mykiss*, Walbaum (Anderson *et al.*, 1997), channel cat fish, *Ictalurus punctatus* Raffinesque (Waterstrat, 1999) and two marine fishes , rabbit fish, *Siganus lineatus* Valenciennes (Soto and Burhanuddin, 1995), *Pomacentrus ambiensis* Bleeker (Munday and Wilson, 1997)

Besides its apparently suitable anaesthetizing properties for fish, clove oil is a relatively inexpensive and user-safe anaesthetic in dentistry that can be used in lower concentrations than other anesthetics such as MS-222(Keene *et al.*, 1998). Additionally fish does not requires a withdrawal period after exposure to the chemical (Anderson *et al.*, 1997)

Soto and Burhanuddin(1995) and Anderson *et al.* (1997) used clove oil concentrations of 33-120mgl^{-1} on rabbit fish *Siganus lineatus* and rainbow trout. Clove oil gave the largest recovery time compared to benzocaine, MS-222, quinaldine, 2- phenoxy ethanol (Munday and Wilson 1997)

2.5.4 Preparation of the anaesthetics

The method used consisted of introducing the active ingredient of clove oil into the fish's gills through water, i.e. 'anaesthesia by immersion'. The substance is absorbed through the gills and travels through the blood stream to central nervous system. The fish then goes through several anaesthesia stages ranging from balance loss to total motionlessness and ventilary arrest (Mc Farland 1960).

Durville and Collet (2002) in their experiment on optimizing the dose of clove oil to be used in anaesthetising wild caught juveniles prepared the

anaesthetic by vigorously shaking a small flask of clove oil and seawater to obtain a whitish emulsion.

Taylor and Roberts (1999) added appropriate dose of clove oil to the water holding container. The water was then agitated by vigorously stirring, after which the fishes were placed in the container.

Giffith(2000) used ethanol to dissolve the clove oil at 1:5 ratios of clove oil and ethanol in his experiment on sampling of the fish assemblages in rock pools.

Successful induction to anaesthesia was determined as the stage where total loss of equilibrium first became evident (i.e. the fish could no longer swim or maintain a vertical position in the water). Concentrations of clove oil was considered suitable if induction to anaesthesia was<3min.(see Marking and Meyer, 1985).

2.5.5 Conditioning

Conditioning for a period of time before packing reduces stress to fish and thus too metabolic rate and fouling during transit. (Richards-Rajadurai 1989).

Prior to transport, fish have to be conditioned so as to reduce stress. A few days before transport, the fish are kept in clear running water in separate tanks. This helps in removing off flavour in the fish. Weak or diseased fish are removed. Feeding is stopped at least 24 hours before transport; this empties the gut of the fish. The temperature of the holding tank is lowered gradually (INFOFISH *Trade News*, 2002).

Conditioning lowers stress, the metabolic rate and oxygen consumption. This keeps the mortality rate low, makes it possible to ship consignments over longer distances and to increase the packing density. A lower metabolic rate reduces the fouling of water by ammonia and carbon dioxide. The packing density of live fish can be greatly increased by proper conditioning as the oxygen demand will be lowered (Shoemaker, 1991).

2.5.6 Ammonia removal

Removal of ammonia can be accomplished by biological means through nitrification by bacteria and ion exchange method with natural zeolite. Clinoptilite a natural zeolite is found to be effective in removing ammonia from water. The effective use of clinoptilite is limited to fresh water application because the ammonia removal capabilities of the natural and synthetic ion exchangers are reduced considerably by competing cations in seawater. Removal of ammonia during transportation of marine fishes in seawater can be carried out by introducing nitrifying bacteria cultured on solid substrate in to the seawater. (Turner and Bower, 1982) Thin pads of polyurethane foam seem to be an ideal substrate for the attachment of ammonia oxidizing bacteria.

2.5.7 Buffers

Rimmer and Franklin (1997) could reduce the carbon dioxide level and increase the pH in the transport medium in a prototype transport system using soda lime and the survival increased from 31% to 100% for barramundi and from 39% to 90% for banded morwong.

Rapid changes in pH stress the fish; hence buffers can be used to stabilize the pH of water during fish transportation. The organic buffer tris-hydroxyl- ethyl- amino- methane is quite an effective in fresh water and seawater (Peer Mohammed and Deavaraj, 1997). Morpholoionopropane sulphonic acid and imidazole have excellent buffer capabilities, but they are toxic to fish in 24 hour exposure (Amend *et al.*, 1982).

3. MATERIALS AND METHODS

3.1 Experimental fish collection and maintenance

Experimental fish, the Malabar grouper (*Epinephelus malabaricus*) was collected from the fishermen who operate near the Cochin barmouth in the back waters from a depth ranging from 2 to 6 m. The fishes were caught very carefully and maintained in cylindrical net cages hung in the water from the boat. The healthy fishes weighing 300g to 700g were collected and transported in 50 l plastic cans well aerated using battery operated aerators to the marine hatchery at CMFRI. After bringing to the hatchery, the fishes were disinfected by providing 200ppm formalin bath for 5 min and were transferred to holding tanks containing seawater. The fish were provided with PVC pipe pieces at the bottom as hide-outs and the holding tanks were well aerated through out. The fishes started feeding in 3 to 4 days time. They were fed with peeled prawn once in a day in the evening. Daily water temperature and salinity were monitored and maintained at 30°C and 35ppt respectively.

3.2 Standardisation of packing procedure

Three preparatory experiments were conducted to standardize the optimal packing requirements – temperature, anaesthetic and biomass to be adopted for further stress ameliorative experiments.

3.2.1 Temperature

Seawater was chilled to 15°C in deep freezer and were packed 3 litre each in polyethylene bags of 10 l capacity inflated with air and tightly closed and packed into 4 boxes containing 1) six numbers of frozen brine bottle alone,

2) six numbers of gel ice sachets alone, 3) three each of frozen brine bottles and gel ice sachets and 4) four sachets of gel ice and two brine bottles. The water temperature was recorded at every 2 h interval.

3.2.2 Anaesthesia

Different concentrations of the two anaesthetics, MS-222 (75 to 400 ppm) and Clove oil (10 to 230 ppm) were prepared and fishes weighing 450±20g were placed in it. The induction and recovery time were noted based on Woody *et al.* 2002.

3.2.3 Packing biomass

Fishes with different body weights (425g, 608g, 796g and 1007g) were oxygen packed in polyethylene bags with 3 l of 15°C 34ppt packing water and packed further in a styrofoam box for 24 h. Survival of fishes were observed along with monitoring of water quality parameters like dissolved oxygen, ammonia and pH.

3.3 Packing materials

3.3.1 Plastic bags

Double lined cylindrical bottom sealed polyethylene bags of 10 l capacities were used as transportation bag.

3.3.2 Polystyrene/styrofoam box

Polystyrene boxes (41.5cm × 26cm × 26cm, thickness 2.5cm) were used for holding the transport bags. The lid was sealed using gummed tape after packing the box with bags containing fish.

3.3.3 Master carton

The polystyrene boxes were packed in corrugated master cartons and were sealed again with gummed tape.

3.3.4 Coolants

Two types of coolants were used, the gel ice sachets and frozen brine bottles. Both were used after freezing for 24-48 h. Gel ice sachets weighed 100g and the brine bottle weighed 400g after freezing. They were used in different numbers and combinations to maintain the temperature around 15°C to 20°C.

3.4 Packing protocol

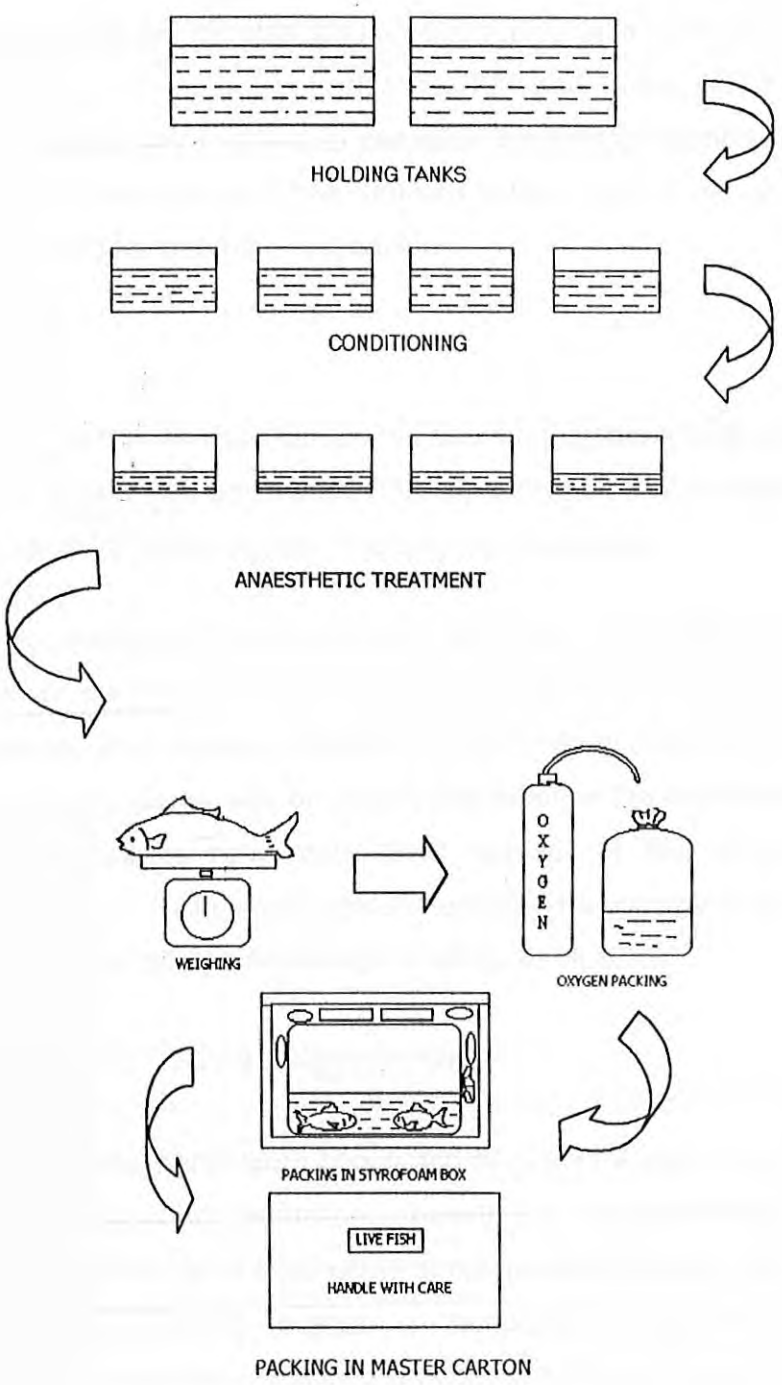
3.4.1 Conditioning

The fishes were provided with clean sea water and starved for 24 h prior to transport. Weak or diseased fish were excluded form the experiments. The tank bottom was siphoned intermittently and 20 to 30 percentage water exchange was done. It was kept well aerated.

3.4.2 Temperature reduction

The fish were transferred from conditioning tanks to 100 l capacity bins in order to facilitate faster temperature reduction with less ice. Four fishes were put in one bin and provided with PVC pipe pieces of 3 to 5 inch size as shelter. Crushed ice, packed in small polyethylene bags (6inch × 10inch size) sealed with rubber bands and checked for any leakage were put into the bins at a rate that will reduce the temperature around 5°C per h. Melted ice bags were replaced with fresh ice bags immediately.

Flow-chart of fish packing steps and procedures



3.4.3 Sedation

5 ml of clove oil was shaken vigorously in a conical flask with 100ml seawater to get a whitish emulsion (Durville and Collet, 2002) of which appropriate quantities were added to the bins containing conditioned fishes after reducing the water to 50 l. The sedated fishes that did not respond to touch were collected for weighing and packing.

3.4.4 Packing

Sedated fishes were introduced into the polythene bags containing 3 l of filtered seawater. The bags were then supersaturated with oxygen under pressure with medical grade oxygen. The bag was tied tightly.

The sealed polyethylene bags with fish were checked for any leakage and were put into the polystyrene box at a rate of one bag per box in horizontal position. The coolants, 4 numbers of gel ice sachets and 2 numbers of frozen brine bottles were place on the top and sides of the polyethylene bag. Care was taken not to have any direct contact of the coolants with transportation water so as to avoid sudden cooling. The box was then covered with the lid and sealed with gummed tape properly at all sides.

3.5 Stress ameliorative experiments

Five experiments were conducted to study the stress ameliorative treatments viz. temperature reduction, anaesthesia, supplementary oxygen packing, combined effects of anaesthesia+ supplementary oxygen packing and anaesthesia + supplementary oxygen + temperature reduction. All the experiments had two replicates. Packing procedure employed was same in all experiments as described under 3.4 Packing protocol.

3.5.1 Effect of temperature on water and blood serum parameters

Two fishes weighing 350±30g each were packed at two different temperatures 30°C and 15°C in eight boxes. The boxes were opened at different time intervals (6h, 12h, 18h and 24h) and noted for survival. Water and blood samples were collected for analysis.

3.5.2 Effect of pre-packing anaesthesia on water and blood serum parameters

Two fishes weighing 350±30g each were packed in 30°C seawater with pre-packing anaesthesia and without pre-packing anaesthesia in five boxes. Water and blood samples were collected at 6 h interval for 24 h.

3.5.3 Effect of oxygen packing on water and blood serum parameters

Two fishes weighing 350±30g each were packed in five boxes with seawater at 30°C with and without additional oxygen. Water and blood samples were collected at 6 h intervals.

3.5.4 Combined effect of anaesthesia + supplementary oxygen packing

The experimental protocol followed was same as in the above experiments. The fishes were anaesthetized in 100ppm clove oil and supplementary oxygen provided and packed in seawater at 30°C along with adequate control group.

3.5.5 Combined effect of anaesthesia + supplementary oxygen packing + lower temperature

The experimental protocol followed was same as in the above experiments. The fishes were anaesthetized in 100ppm clove oil; supplementary

oxygen provided and packed in seawater at 15°C along with adequate control group.

3.6 Sampling protocol

3.6.1 Water sample collection and analysis

Water samples were collected at 6h intervals for dissolved oxygen, ammonia and pH estimations. Dissolved oxygen was estimated using the Winkler method (Strickland and Parsons, 1972) and the ammonia using phenol-hypochlorite spectrophotometric method (Strickland and Parsons, 1972). pH was estimated using a digital pH meter.

3.6.2 Blood sample collection and analysis

The fishes were restrained by keeping a piece of cloth on the head for better grip and to avoid loosing of slime. 19 gauge needle with 2ml Dispovan syringe was introduced to the caudal vein of the fish (Schaperclaus, 1986) and the piston was moved to drive in the blood held in a manner that gives gravity advantage for the blood to flow into the syringe. Around 1.5ml of blood was drawn and transferred to small glass test tubes and were left for clotting at room temperature for one hour. The test tubes were then plugged with cotton and stored in the refrigerator. Serum was separated from this and stored in the eppendorf's tubes in freezer for further analysis.

Glucose (Raabo 1969) and alkaline phosphatase (Kind and King 1954) were estimated using Qualigens diagnostic kits. Total protein was estimated using Lowry's method (Lowry *et al.*, 1951).

3.7 Statistical analysis

The values obtained were statistically analysed by the method of one way analysis of variance in computer software Systat (SPSS. INC., 1997).

4. RESULTS

4.1 Maintenance of water temperature

A combination of frozen gel ice sachets and brine bottle was found effective in keeping the seawater (35 ppt.) temperature in the containers at the optimal range (15-20°C) based on the results of the experiment conducted to study the efficacy of different coolants in maintaining water temperature (Table 1). When the frozen brine bottles alone (6 No) were used the temperature dropped considerably to as low as 9.2°C. When gel ice sachets alone were used, the temperature increased steadily to reach a final temperature of 25°C at 24th hour. The gel ice and brine bottle combination of 3 each gave better temperature maintenance (15°C to 20°C) for 24h. When the number of brine bottle was reduced to 2 and gel ice increased 4 sachets, the final temperature shot up to 23.2°C.

Table.1 Changes in water temperature over time in the container when coolants were used singly and in combinations

Time (h)	Temperature °C			
	Brine Bottle-6 No	Gel Ice-6 No	Brine Bottle-3 Gel ice-3	Brine Bottle-2 Gel ice-4
00	15.0	15.0	15.0	15.0
02	11.7	14.0	13.0	14.2
04	10.8	13.0	13.5	14.0
06	10.2	13.2	12.7	13.2
08	9.7	13.8	13.2	13.7
10	9.5	14.5	13.9	14.3
12	9.7	15.7	14.2	14.9
14	9.2	16.7	15.2	15.7
16	9.7	18.0	16.3	17.1
18	9.9	19.2	17.1	18.2
20	10.5	21.4	17.9	19.5
22	11.2	23.2	18.6	21.1
24	13.2	25.0	20.1	23.2

4.2 Induction and duration of anaesthesia

The induction time and duration of anaesthesia for various doses of clove oil and MS-222 are shown in Tables 2 and 3. Clove oil induced anaesthesia at lower doses compared to MS-222. A dose of 10 ppm clove oil induced anaesthesia in 5.4 min and the lowest dose of MS-222 used here (75ppm) did not induce anaesthesia in 10 min time. 90 ppm clove oil induced anaesthesia in 190 sec and MS-222 dose required for the same time of induction was near 200 ppm.

Higher doses of clove oil (100 ppm to 230 ppm) gradually reduced the induction time while the induction time decreased rapidly with increase (75-400 ppm) in dose of MS-222. The highest dose of MS-222 (400 ppm) induced anaesthesia in 95 sec (Table 3).

The duration of anaesthesia was considerably less for the MS-222 treated fishes especially at lower doses. Anaesthesia duration of only 50 seconds was possible with 100ppm of MS-222 but clove oil dose as low as 10ppm kept fishes anaesthetised for 70 seconds. The duration of anaesthesia increased steadily with increase in dose of clove oil.

Table 2 Induction time and duration of anaesthesia when clove oil is used as anaesthetic

Dose (ppm)	Induction Time (sec.)	Anaesthesia Duration (sec.)
10	325	70
30	300	100
50	245	200
70	200	260
90	190	300
110	165	330
130	150	345
150	145	360
170	138	372
190	123	380
210	124	395
230	120	400

Table. 3. Induction time and duration of anaesthesia when MS-222 is used as anaesthetic

Dose (ppm)	Induction Time (sec.)	Anaesthesia Duration (sec.)
75	--	--
100	370	50
125	290	80
150	250	93
175	215	102
200	180	137
225	175	153
250	150	189
275	141	220
300	130	280
325	115	320
400	95	350

4.3 Effect of biomass on the water quality parameters

Fishes in the pack with biomass upto 796 g survived a 24 h holding time while those in the pack with biomass of 1007 g were found dead. The water quality parameters recorded at 24th h are shown in the Table 4. Dissolved oxygen decreased to a very low level of 1.344 mgL^{-1} and ammonia level increased to 12.94 ppm from an initial value of 0.271 ppm in the packs with biomass of 1007 g. The pH decreased to 5.95 from an initial of 8.5 (Table 4).

Table.4 Water quality parameters in bags with different biomass.

Weight (g)	DO (mgL^{-1})		Ammonia (ppm)		pH		Temperature (°C)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
425	7.85	5.380	0.271	6.080	8.50	7.25	15.0	22.1
608	7.85	4.284	0.271	7.180	8.50	7.05	15.0	21.2
796	7.85	2.320	0.271	10.284	8.50	6.22	15.0	21.9
1007	7.85	1.344	0.271	12.994	8.50	5.95	15.0	21.4

4.4 Effect of temperature on water quality and serum parameters

Water quality and serum parameters are recorded for 0, 6 and 12 h for both control (30°C) and treatment (15°C) as the fishes did not survive > 12 h.

Water quality parameters are presented in the Table 5, Figure 1, 2&3. Dissolved oxygen decreased from 7.02±0.22mgL^{-1} at zero h to 0.63 ± 0.09 mgL^{-1} at 12th h in the treatment and 0.53±0.79 mgL^{-1} in the control. Ammonia values increased to 11.33 ± 2.11 ppm in the control and 09.41 ± 1.72 ppm in the treatment from an initial value of 0.09 ± 0.01 ppm. pH

decreased to a very low of 4.31 ± 0.57 at 12th h in the control and 4.91 ± 1.58 in the treatment from an initial of 8.05 ± 1.84. Test using one-way ANOVA indicated that there is no significant difference ($p > 0.05$) in the water quality parameters in the control and treatment.

All the serum parameters showed an increasing trend with time, but the values remained higher in the control fishes at all points of time. There was a significant difference ($p < 0.05$) in the case of serum glucose and alkaline phosphatase but significant difference ($p > 0.05$) was not observed in the case of total protein (Table 6, Figures 4, 5&6).

4.5 Effect of anaesthesia on water quality and serum parameters

Fishes subjected to pre-packing anaesthesia (Treatment) survived for 18 hours and those unanaesthetised prior to packing (Control) did not survive. Hence the water and serum parameters are not recorded for 18th hour in the control.

Dissolved oxygen decreased from 7.02 ± 0.22 mgL^{-1} at zero hour to 0.53 ± 0.79 mgL^{-1} in the control group and 1.92 ± 1.10 mgL^{-1} in the treatment group. Ammonia values were 0.09 ± 0.01ppm initially and it increased to 8.75 ± 1.79 ppm in the treatment and 11.33 ± 2.11 ppm in the control by 12 h. Ammonia concentration was 10.58 ± 2.11 in the treatment group at 18th h. pH decreased from initial value of 8.05 ± 1.84 to 4.5 ± 0.57 in the control and 6.91 ± 2.10 in the treatment by 12 h (Table 7; Figures 7,8&9). Significant difference ($p < 0.05$) was noted in the dissolved oxygen and ammonia values while difference was not significant ($p > 0.05$) for pH.

Serum parameters showed increasing trend. Increase was sharp in the case of fishes in the control group. Serum glucose increased from an initial value of 7.93 ± 0.06 mg% to 12.89 ± 0.99 mg% in the treatment and 19.91 ± 2.50 mg% in the control fishes in the 6th h. It increased to 17.76 ± 1.71 mg% and 21.51 ± 0.99 mg% respectively for treatment and control by 12 h.

Alkaline phosphatase level increased to 18.95 ± 0.30 KA units in treatment and 22.04 ± 2.11 KA units in the control fishes from an initial level of 7.05 ± 0.39 KA units by 12 h. ALP was 25.03 ±0.99 KA units at 18th h in the treatment fishes. Total protein level increased from an initial of 3.03 ± 0.50 mg% to 5.01 ± 0.81 mg% in the treatment and 7.31 ± 0.61 mg% in fishes of the control group at 12th h (Table 8; Figures 10,11&12). Significant differences ($p < 0.05$) were noted for all the parameters.

4.6 Effect of oxygen packing on the water quality and serum parameters

Fishes in the oxygen packed containers (Treatment) survived for 24 h while those packed without oxygen (Control) survived only for 12 h. The water and serum parameters were recorded up to 12th h only in the case of control.

Dissolved oxygen was 7.02 ± 0.22 mgL^{-1} initially and it decreased over time to 2.41 ± 1.10 mgL^{-1} in the control and to 6.05 ± 2.11 mgL^{-1} in the treatment by 6 h. It further decreased to 0.53 ± 0.79 mgL^{-1} in the control while DO was 4.91 ± 1.88 mgL^{-1} in the treatment at 12th h. The DO was 2.10 ± 1.81 mgL^{-1} in the treatment at the end of 24 h. Ammonia level increased to 11.33 ± 2.11 ppm in the control and to 9.02 ± 2.24 ppm in the treatment at 12 h from an initial of 0.091 ± 0.01 ppm. pH decreased to 4.8 ± 0.57 in the control and to 5.21 ± 1.01 in the treatment (Table 9; Figure 13, 14&15). DO and ammonia values were significantly different ($p < 0.05$) while the pH was not significantly different ($p > 0.05$).

Serum glucose increased from 7.93 ± 0.06 mg% at zero hour to 21.51 ± 0.99 mg% in the control and 19.02 ± 0.07 in the treatment fishes by 12 h. Glucose value remained at 22.11 ± 0.47 mg% in the treatment fishes at 24th h. ALP values were 7.17 ± 0.47 KA units initially and increased over time to 22.04 ± 2.11 in the control fishes and 21.03 ± 0.31 KA units in the treatment fishes. Total protein level increased 7.31 ± 0.61 mg% in the control and 7.23 ± 0.91 mg% in the treatment (Table 10; Figure 16, 17&18). While the glucose and alkaline phosphatase values were significant ($p < 0.05$), the total protein values were not significant ($p > 0.05$).

Table 5 Effect of temperature on water quality parameters

Duration (h)	Treatments	DO (mgL^{-1})	Ammonia (ppm)	pH
0	30°C	7.02±0.22	0.091±0.01	8.05±1.84
	15°C	7.02±0.22	0.091±0.01	8.05±1.84
6	30°C	2.41±1.10	09.21±0.88	5.21±1.59
	15°C	3.21±1.24	07.53±1.56	6.71±2.10
12	30°C	0.53±0.79	11.33±2.11	4.31±0.57
	15°C	0.63±0.09	09.41±1.72	4.91±1.58

DO- Dissolved Oxygen, ALP- Alkaline Phosphatase, TP- Total Protein, KA- King Amstrong Unit.

Table 6 Effect of temperature on serum parameters

Duration (h)	Treatments	Glucose (mg%)	ALP (KA unit)	TP (mg%)
0	30°C	7.93±0.06	7.17±0.47	3.03±0.50
	15°C	7.93±0.06	7.17±0.47	3.03±0.50
6	30°C	19.91±2.50	21.11±1.77	5.10±0.38
	15°C	16.81±1.67	16.52±0.99	4.21±0.41
12	30°C	21.51±0.99	22.04±2.11	7.31±0.61
	15°C	20.30±0.99	18.19±0.89	7.23±0.95

Table 7 Effect of anesthesia on water quality parameters

Duration (h)	Treatments	DO (mgL^{-1})	Ammonia (ppm)	pH
0	Anaesthetised	7.02±0.22	0.091±0.01	8.05± 1.84
	Unanaesthetised	7.02±0.22	0.091±0.01	8.05± 1.84
6	Anaesthetized	3.21±0.55	6.92±3.10	7.12 ±1.84
	Unanaesthetised	2.41±1.10	9.21±0.88	5.21 ±1.59
12	Anaesthetised	1.92±1.10	8.75±1.79	6.91± 2.10
	Unanaesthetised	0.53±0.79	11.33±2.11	4.51± 0.57
18	Anaesthetised	0.92±0.67	10.58±2.11	5.31± 1.39
	Unanaesthetised	--	--	--
24	Anaesthetised	0.31±0.13	12.01±1.99	4.37± 1.52
	Unanaesthetised	--	--	--

Table 8 Effect of anesthesia on blood serum parameters

Duration (h)	Treatment	Glucose (mg%)	ALP (KA unit)	TP (mg%)
0	Anaesthetised	07.93±0.06	07.05±0.39	3.03±0.50
	Unanaesthetised	07.93±0.06	07.17±0.47	3.03±0.50
6	Anaesthetised	12.89±0.99	15.32±0.19	3.91±0.69
	Unanaesthetised	19.91±2.50	21.11±1.77	5.10±0.38
12	Anaesthetised	17.76±1.71	18.95±0.30	5.01±0.81
	Unanaesthetised	21.51±0.99	22.04±2.11	7.31±0.61
18	Anaesthetised	19.21±2.07	25.03±0.99	7.02±1.10
	Unanaesthetised	--	--	--

Table 9 Effect of oxygen packing on water quality parameters

Duration (Hrs.)	Treatments	DO (mgL^{-1})	Ammonia (ppm)	pH
0	Oxygen packing	7.02±0.22	0.091±0.01	8.05±1.84
	No oxygen packing	7.02±0.22	0.091±0.01	8.05±1.84
6	Oxygen packing	6.05±2.11	7.11±1.40	6.01±1.39
	No oxygen packing	2.41±1.10	9.21±0.88	5.21±1.59
12	Oxygen packing	4.91±1.88	9.02±2.24	5.21±1.01
	No oxygen packing	0.53±0.79	11.33±2.11	4.83±0.57
18	Oxygen packing	3.25±1.61	10.92±0.99	5.00±0.99
	No oxygen packing	--	--	--
24	Oxygen packing	2.10±1.81	12.98±1.94	4.22±1.25
	No oxygen packing	--	--	--

Table 10 Effect of oxygen packing on blood serum parameters

Duration (Hrs.)	Treatments	Glucose (mg%)	ALP (KA unit)	TP (mg%)
0	Oxygen packing	7.93±0.06	7.17±0.47	3.03±0.50
	No oxygen packing	7.93±0.06	7.17±0.47	3.03±0.50
6	Oxygen packing	17.81±0.07	20.51±0.57	5.05±0.81
	No oxygen packing	19.91±2.50	21.11±1.77	5.10±0.38
12	Oxygen packing	19.02±0.07	21.03±0.31	7.23±0.91
	No oxygen packing	21.51±0.99	22.04±2.11	7.31±0.61
18	Oxygen packing	20.56±0.26	23.11±0.66	8.27±0.81
	No oxygen packing	--	--	--
24	Oxygen packing	22.11±0.47	25.65±0.99	9.95±1.31
	No oxygen packing	--	--	--

Figure 1. Effect of temperature on dissolved oxygen

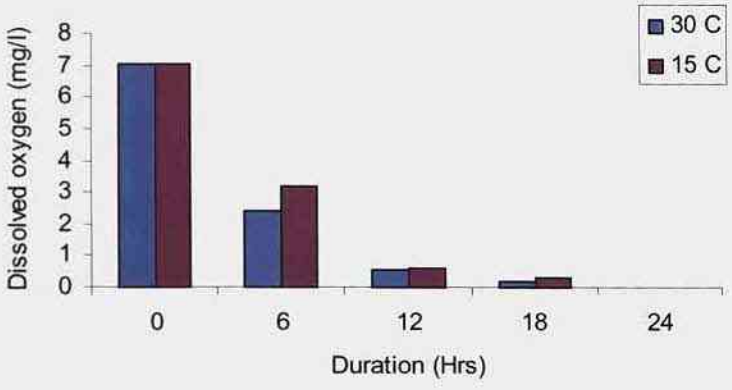


Figure 2. Effect of temperature on Ammonia

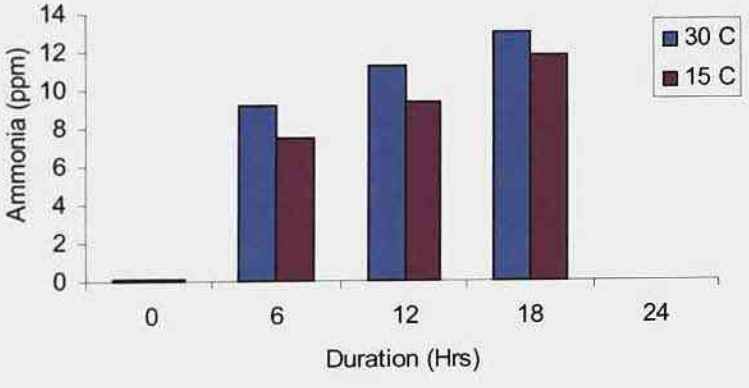


Figure 3. Effect of temperature on pH

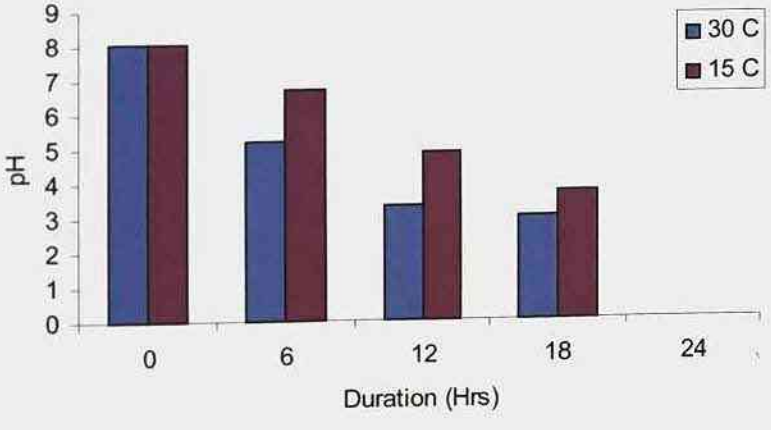


Figure 4. Effect of temperature on serum glucose

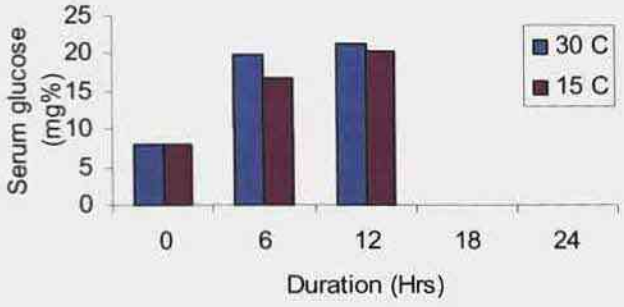


Figure 5. Effect of temperature on serum alkaline phosphatase

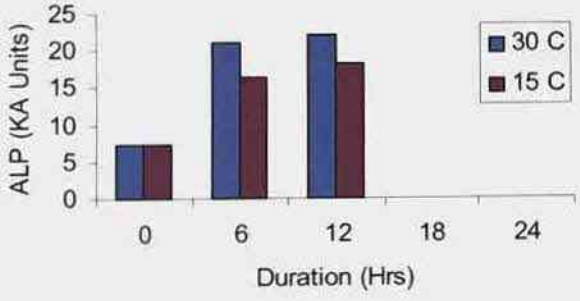


Figure 6. Effect of temperature on serum total protein

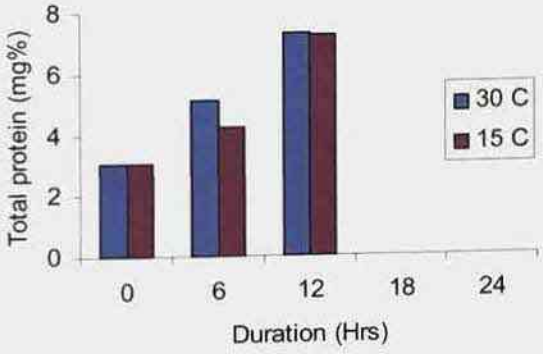


Figure 7. Effect of anaesthesia on dissolved oxygen

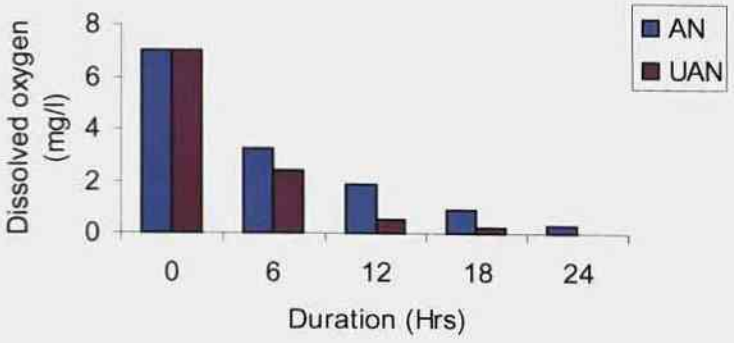


Figure 8. Effect of anaesthesia on Ammonia

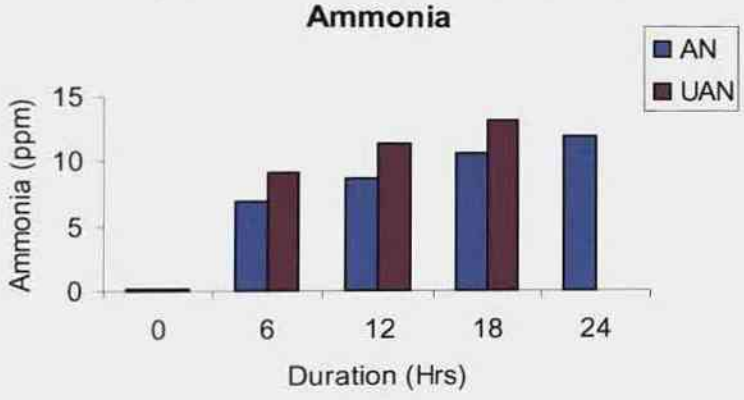


Figure 9. Effect of anaesthesia on pH

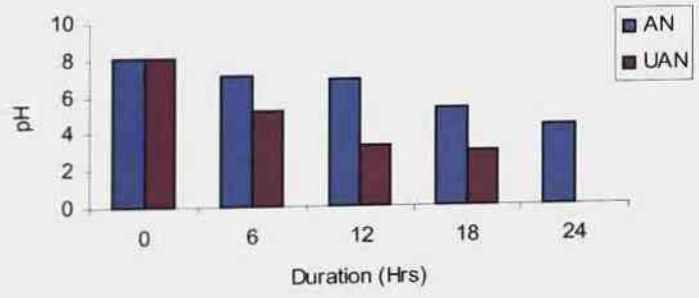


Figure 10. Effect of anaesthesia on serum glucose

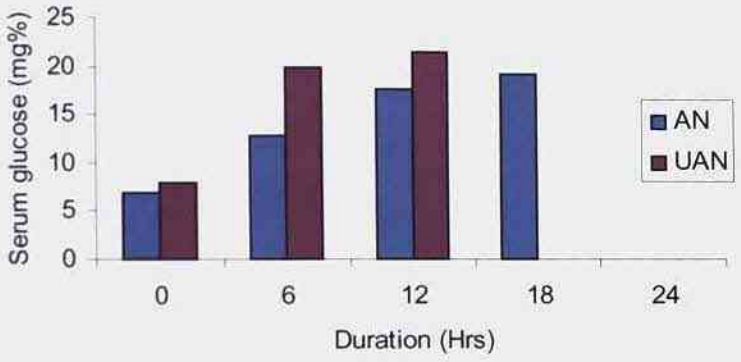


Figure 11. Effect of anaesthesia on serum alkaline phosphatase

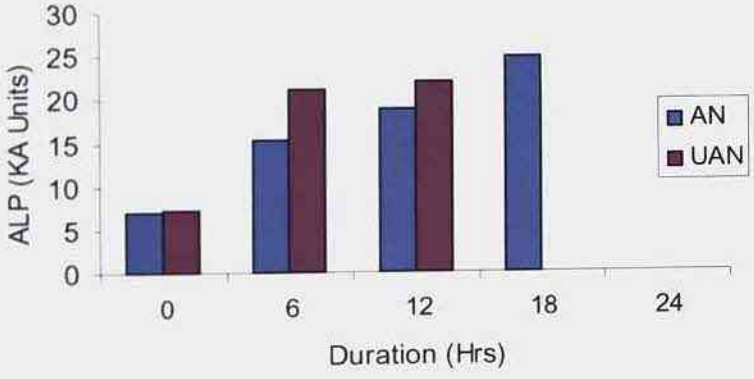


Figure 12. Effect of anaesthesia on serum total protein

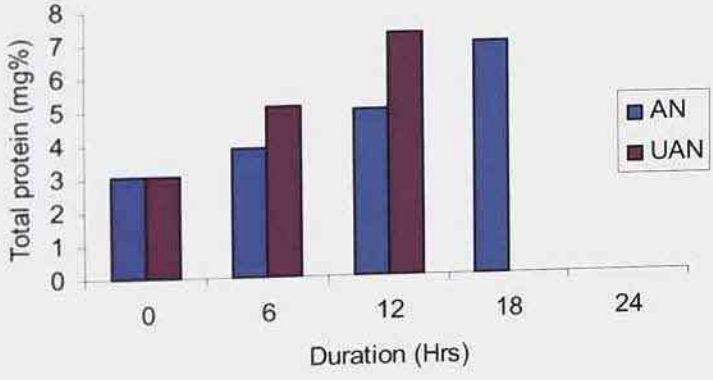


Figure 13. Effect of oxygen packing on dissolved oxygen

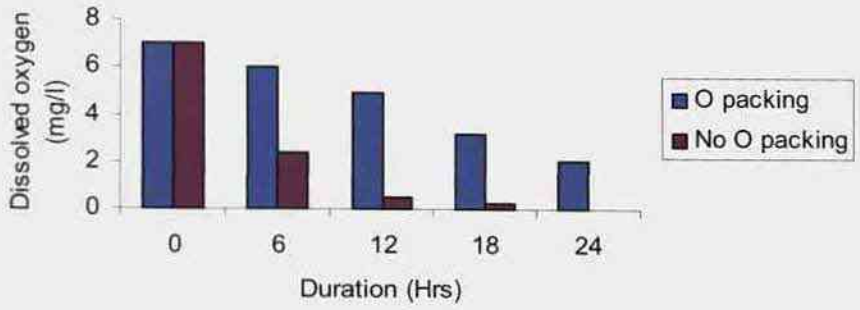


Figure 14. Effect of oxygen packing on ammonia

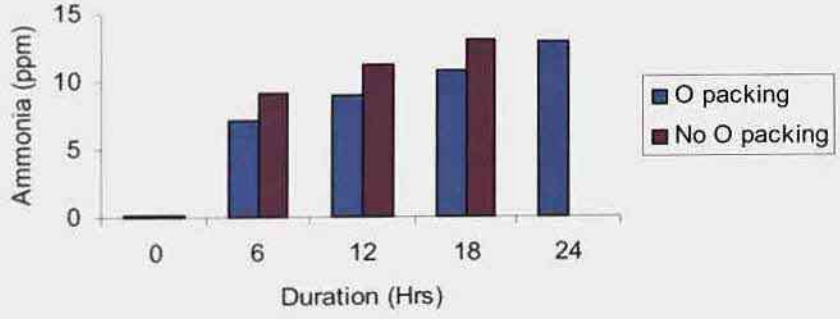


Figure 15. Effect of oxygen packing on pH

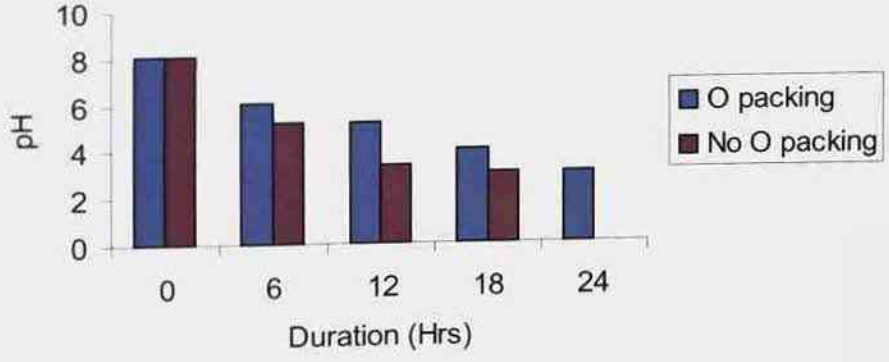


Figure 16. Effect of oxygen packing on serum glucose

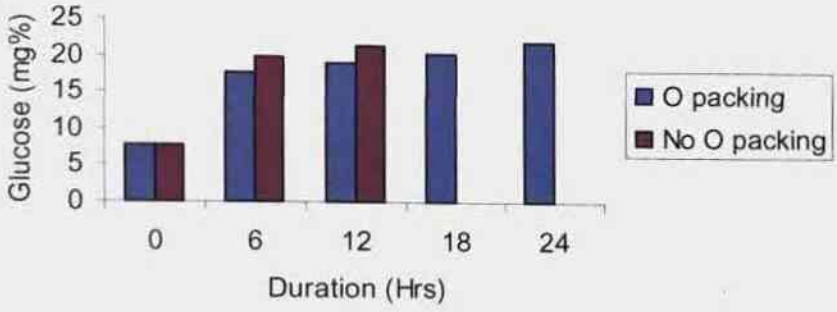


Figure 17. Effect of oxygen packing on serum alkaline phosphatase

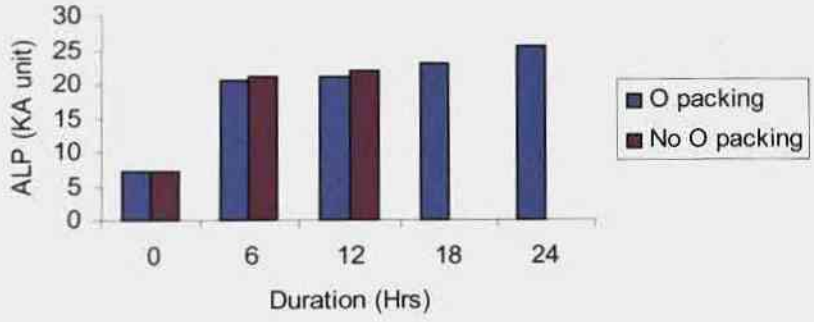
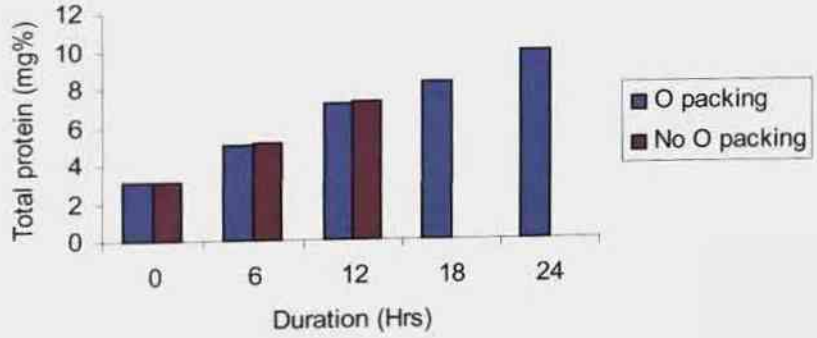


Figure18. Effect of oxygen packing on serum total protein



4.7 Combined effect of anaesthesia + supplementary oxygen packing

Water quality parameters at different times in anaesthetised and oxygen packed (Treatment) and unanaesthetised and no oxygen packed (control) are presented in Table 11A and 11B.

DO values were 7.02 ± 0.22 mg/L^{-1} at zero h and decreased to 2.13 ±1.10 mgL^{-1} in anaesthetized and oxygen packed and 0.21 ± 0.09 mgL^{-1} in control group. DO was 1.06 ± 0.88 mgL^{-1} at 24th hour in treatment group and it was not recorded for the control group as they did not survive beyond 12 h. Ammonia values were 0.09 ± 0.01 ppm at zero h and it increased to 10.45 ± 2.16 ppm in treatment group and to 13.13 ± 3.6 ppm in the control group at the end of 18 h and pH decreased to 5.05 ± 2.0 in treatment from a mean of 8.05 ± 1.95 at zero h.

Serum glucose were 7.93 ± 0.06 mg % at zero h and increased to 14.51 ± 0.10 mg % in treatment fishes and 21.51 ± 0.99 mg % in control fishes by 12 h. The glucose level was 20.91 ± 0.17 mg % at 24th hour in anaesthetised and oxygen packed fishes. ALP was 7.17 ± 0.47 KA units at zero h. It increased sharply in first 6 hours to 17.31 ± 0.17 KA units in treatment group and to 21.11 ± 1.77 KA units in control fishes. It increased further to 19.31 ± 0.29 KA units in treatment fishes and 22.04±2.11KA units in the control fishes in 12 h. The ALP value was 23.12 ± 1.17 KA unit at the end of 24 h in the treatment fishes. TP was 3.03 ± 0.50 mg % at zero h and increased gradually to 5.81 ± 0.71 in treatment fishes and 7.31 ± 0.61 mg % in control fishes by 12th h. The TP value was 8.75 ± 1.07 mg % at 24th h in anaesthetised and oxygen packed fishes.

Table11 A Effect of anesthesia and oxygen packing on water quality parameters at 30 °C

Duration (h)	Anaesthetized oxygen packed		
	DO (mgL^{-1})	NH_3 (ppm)	pH
0	7.02±0.22	0.09±0.01	8.05±1.94
6	6.21±0.17	6.21±0.89	7.01±1.90
12	3.72±0.98	8.12±1.78	6.30±1.89
18	2.13±1.10	10.45±2.16	5.05±2.00
24	1.06±0.88	11.17±2.09	4.37±1.62

Table 11 B Effect of anesthesia and oxygen packing on water quality parameters at 30 °C

Duration (h)	Unanaesthetized no-oxygen packing		
	DO (mgL^{-1})	NH_3 (ppm)	pH
0	7.02±0.22	0.091±0.01	8.05±1.84
6	2.41±1.10	9.21±0.88	5.21±1.59
12	0.53±0.79	11.33±2.11	4.51±0.57

Table 12 A Effect of anesthesia and oxygen packing on serum parameters at 30°C

Duration (h)	Anaesthetized oxygen packed		
	Glucose (mg%)	ALP (KA Unit)	TP (mg%)
0	7.93±0.06	7.17±0.47	3.03±0.50
6	12.32±0.08	17.31±0.17	4.02±0.37
12	14.51±0.10	19.31±0.29	5.81±0.71
18	17.21±0.07	21.10±0.34	7.05±1.01
24	20.91±0.17	23.12±1.17	8.75±1.07

Table 12 B Effect of anesthesia and oxygen packing on serum parameters at 30°C

Duration (h)	Unanaesthetized and no-oxygen packed		
	Glucose (mg%)	ALP (KA Unit)	TP (mg%)
0	7.93±0.06	7.17±0.47	3.03±0.50
6	19.91±2.50	21.11±1.77	5.10±0.38
12	21.51±0.99	22.04±2.11	7.31±0.61

4.8 Combined effect of anaesthesia + supplementary oxygen packing + lower temperature

Fishes in the anaesthetised and oxygen packed (Treatment) survived for 24 h but unanaesthetised and no oxygen packed fishes (Control) survived for 12h.

The water quality parameters are reported in Table 13 A and Table 13B. DO values were 7.02 ± 0.22mg/L^{-1} at zero h and decreased over time to 5.12 ± 0.56mg/L^{-1} in treatment group and to 0.28 ± 0.21mg/L^{-1} in control group at the end of 18th hour. The DO was 3.98 ± 0.71 mg/L^{-1} in treatment group at 24th h. Ammonia values were 0.09 ± 0.01 ppm at zero h and it increased to 8.53 ± 1.88 ppm in treatment group and 11.95 ± 1.88 ppm in the control group at 18th h. The ammonia was 10.01 ± 1.92 ppm at 24th h in anaesthetised and oxygen packed fishes. pH was 8.05 ± 1.84 at zero h and gradually decreased over time to 6.11 ± 1.5 in treatment group fishes and 3.71 ± 0.89 in control.

Serum parameters were recorded upto 12th h in control. Glucose was 7.93 ± 0.06 mg % at zero h and increased to 12.59 ± 0.06 mg % in treatment fishes and to 20.3 ± 0.99 in control fishes at 12th h. The glucose values in the treatment fishes were 18.2 ± 0.24 mg % at 24th h. Mean ALP at zero h was 7.17 ± 0.47 KA and increased over time to 18.19 ± 0.89 KA unit in the control fishes and that in the treatment fishes were 15.91 ± 0.41 KA. The ALP value in the treatment fishes were 20.03 ± 1.77 KA unit at 24th h. Mean TP value at zero h was 3.03 mg %. It increased to 4.21 ± 0.66 mg % in treatment fishes and 7.23 ± 0.95 mg % in control fishes by 12 h. The TP value was 5.21 ± 1.10 mg % at 24th h in anaesthetised and oxygen packed fishes.

Table13 A Effect of anesthesia and oxygen packing on water quality parameters at 15°C

Duration (h)	Anaesthetized oxygen packed		
	DO (mg/L)	NH_3 (ppm)	pH
0	7.02±0.22	0.091±0.01	8.05±1.84
6	7.03±1.57	4.910±0.69	7.57±1.67
12	6.98±1.47	6.230±1.26	6.95±0.44
18	5.12±0.61	8.530±1.71	6.11±0.71
24	3.98±0.51	10.010±2.14	5.94±1.12

Table13 B Effect of anesthesia and oxygen packing on water quality parameters at 15°C

Duration (h)	Unanaesthetized no-oxygen packing		
	DO (mg/L)	NH_3 (ppm)	pH
0	7.02±0.22	0.091±0.01	8.05±1.84
6	3.21±1.24	7.530±1.72	6.71±2.10
12	0.63±0.09	9.410±1.88	4.91±1.58

Table 14 A Effect of anesthesia and oxygen packing on serum parameters at 15°C

Duration (h)	Anaesthetized and oxygen packed		
	Glucose (mg%)	ALP (KA Unit)	TP (mg%)
0	7.93±0.06	7.17±0.47	3.03±0.50
6	10.33±0.05	14.05±0.27	3.45±0.77
12	12.59±0.06	15.91±0.41	4.21±0.66
18	14.23±0.11	17.33±0.51	4.88±0.31
24	18.2±0.24	20.03±1.77	5.20±1.10

Table 14 B Effect of anesthesia and oxygen packing on serum parameters at 15°C

Duration (h)	Unanaesthetized and no-oxygen packing		
	Glucose (mg %)	ALP (KA Unit)	TP (mg %)
0	7.93±0.06	7.17±0.47	3.03±0.50
6	16.81±1.67	16.52±0.99	4.21±0.41
12	20.3±0.99	18.19±0.89	7.23±0.95

5 DISCUSSION

Transportation procedures including initial capture, loading into containers, the actual transport, unloading and stocking induce stress in fish. High packing density and poor water quality may be additional stressors. A variety of methods have been used to ameliorate the adverse effects of stress on fish during live transportation. Marked difference exists in the efficacy of these treatments in different species and information regarding optimal long duration transport requirements especially for marine fish species is insufficient. There is no approved procedure available for live transport of grouper for human consumption.

Three preparatory experiments were conducted to standardize the packing water temperature, level of anaesthesia and biomass carrying capacity to be followed uniformly in subsequent experiments. On the basis of the results of the above experiments, 3 other experiments were carried out to study the effect of low temperature, pre-packing anaesthesia and additional oxygen to reduce the adverse impacts of stress on transport. Changes in water parameters of the transporting medium and blood chemistry of the fish were considered as indicative response parameters. Further two experiments undertaken to study the combined effects of anaesthesia, supplementary oxygen packing and temperature reduction in live transport of *Epinephelus malabaricus*. The results have been tabulated and graphically presented.

5.1 Packing water temperature

It is well known that metabolic rate is influenced by environmental factors particularly temperature and dissolved oxygen. Neill and Bryan (1991) considered metabolic scope to be an integrative measure of environmental quality for aquaculture. High metabolic rate, commensurate with high water temperature as observed in turbot *Scopthalmus maximus* (L) (Claireaux and Lagardere, 1999). Fish absorb oxygen and release ammonia besides carbon dioxide, which are

deleterious for the survival of the fish. Lowering the temperature can reduce the metabolic activity; thereby oxygen can be conserved for prolonged use while detrimental build up of gases, metabolites and in turn high fluctuation in pH can be minimized.

Most of the tropical fishes cannot tolerate lower temperatures below 12°C (Rajadurai, 1989). The ideal temperature for transport will be 15°C to 20°C. A combination of gel ice sachets and brine bottles used in the present study were found to be effective in keeping the water temperature at optimal range for 24 h (Table 1).

5. 2 Induction and duration of anaesthesia

During initial handling and packing, the metabolic rate increases drastically due to struggling and excitement. It has been indicated that deep sedation suppresses reactivity of fish to external stimuli without upsetting equilibrium and reduce oxygen consumption of individuals to basal rates (McFarland, 1959). Reese (1953) suggested that sodium amytal was beneficial for trout in that it might reduce the activity of fishes and result in slowing down their body functions and oxygen consumption.

The use of anaesthetics on food fish is very limited. Two anaesthetics considered safe for use on food fish are MS-222 (3-aminobenzoic acid ethyl ester methanesulphonate) and carbon dioxide (CO_2) (Schnick *et al.*, 1979). The anaesthetic MS-222 is effective but has limited use because some regulatory agencies such as the U.S. Food and Drug Administration (FDA) require that fishes treated with MS-222 are held for minimum 21 days before human consumption. Various derivations of CO_2 used to anaesthetize fishes are generally recognized as safe for human intake, but are considered only partly effective by many biologists. Alternate anaesthetics for food fishes, which are effective, would fill a priority need in fisheries science (Gilderhus and Marking, 1987).